****

**Lab Seven: Neuromodulation**

**Overview**

**In this lab you will:**

1. learn how to extract neuromodulating agents from common products;
2. test how these agents affect neuronal spiking patterns; and
3. investigate how different classes of neuromodulators affect various populations of neurons in the cricket.

**Objectives**

**Before doing this lab you should understand:**

* the basics of cricket anatomy
* how neuromodulators affect a synapse
* how the differential expression of receptors can alter neuronal responsiveness

**After doing this lab you should be able to:**

* explain neuropharmacology and neuromodulation
* describe how alcohol, glutamate, and nicotine affect neuronal firing rates
* design an experiment to test how a substance may affect the nervous system

**EQUIPMENT**

* SpikerBox with audio cable adapter
* computer with Audacity installed
* crickets
* ice water or access to a freezer
* dissection scissors
* toothpick
* 3” petri dish
* corrugated cardboard (cut to fit into petri dish)
* plastic pipettes
* distilled H20, NaCl, baking soda, MSG, ethanol, tobacco
* 1 cc (1 ml) syringes

**Introduction**

****

**About Anuradha Rao**

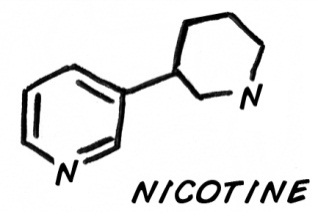
This experiment is dedicated to Anuradha Rao, a neuroscientist who studied pharmacology and enjoyed educational outreach. Her memorial fund generously allowed Backyard Brains to present experiments and prototypes at the 2010 Society for Neuroscience Conference in San Diego, CA.

In this experiment we will test the effect of neuroactive compounds on central nervous system neurons. Obtaining drugs that affect neurons can be quite difficult, as they are often very dangerous (like the batrachotoxins of poison dart frogs or the tetrodotoxins of fugu puffer fish, both of which block sodium channels) or are drugs of abuse (like cocaine, which allows dopamine to stay in synapses longer than normal).

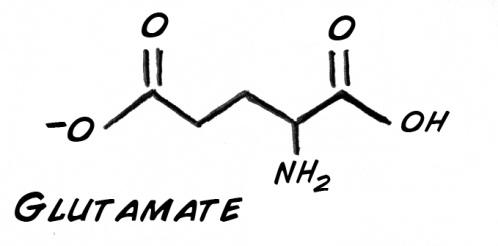
Also, the blood-brain barrier (BBB) prevents the passive diffusion of many substances into the brain. The BBB is composed of brain endothelial cells that are packed closely together around all capillaries, with tight junctions and a higher than normal electrical resistance (2000 Ω/cm2 as opposed to the usual 5 – 10 Ω/cm2). This barrier ensures brain function in vertebrates and insects by maintaining the ionic integrity inside the brain. It prevents many toxic substances from entering the brain, protects brain neurons from circulating neurotransmitters such as norepinephrine, and restricts the diffusion of objects such as bacteria or hydrophilic molecules into the cerebrospinal fluid (CSF). Even K+ ions are excluded. The barrier does allow the diffusion of small hydrophobic molecules (e.g. O2, some hormones), and specialized barrier cells actively transport some metabolic products such as glucose across the BBB with specific proteins. The practical consequence of the BBB on our experiments is that only certain drugs with specific characteristics can be used to influence neurons. Those also makes designing drugs to treat brain function very difficult for pharmaceutical companies.

BBB image courtesy of Wikipedia

However, we do have access to some neuroactive compounds that we can use on our insects: nicotine, monosodium glutamate, and ethanol.



Nicotine comes from the tobacco plant. Tobacco evolved nicotine to prevent insects from eating its leaves. Nicotine is a powerful acetylcholine receptor **agonist**; it amplifies the effect of acetylcholine (ACh) binding to its receptors in synapses, causing a neuron to fire more (due to increased sodium ion influx).

Whereas nicotine is a drug that acts on receptors that neurotransmitters bind to, monosodium glutamate itself is a neurotransmitter. Once dissolved in water, it turns into positively charged sodium ions and negatively charged glutamate ions. Glutamate is normally part of the metabolic pathway of glycolysis (breakdown of sugar) and is readily available from the foods you eat.

In fact, over 80% of the synapses in your brain use glutamate as it is an excitatory neurotransmitter. Do you think glutamate will be excitatory in an insect as well?

Ethanol is a drug that also is an agonist to neurotransmission, however it acts to increase the effect of GABA binding channels. Unlike ACh receptors, stimulation of GABA channels often leads to inhibition of action potentials. In other words, GABA channel stimulation will slow down the firing of a neuron. Stimulation of human neurons with ethanol can slow them down; will the same happen in an insect?

**PROCEDURE**

**Exercise 1: Preparation of Neuroactive Solutions**

**Saline Solution:** Create a simple saline solution by combining 1.5 g table salt (NaCl) and 1.25 g baking soda in 250 mL of distilled water.

**Nicotine:** To create your nicotine solution, take a cigarette or small cigar, remove all the shredded tobacco leaves, and place them in a small container (a clear pill bottle, for example). Fill the container with saline solution, put the cap on, shake up the mixture, and allow it to sit for a couple days to extract the nicotine. Over time the solution should turn yellowish-brown. If you are in high school, your teacher may have already prepared this solution.

**Glutamate:** To create your glutamate solution, you will use the food additive monosodium glutamate (MSG). You can find this at an Asian Import grocery store, as MSG is a common flavor enhancer in Asian foods. Fill up a clear pill bottle about a quarter full of the MSG salt crystals, fill the remainder of the bottle with saline solution, and shake thoroughly to dissolve the MSG. Note that not all of the MSG will dissolve, as you are making a saturated solution.

**Ethyl Alcohol:** Mix 196 mL of saline with 4 mL ethanol to make a **2% EtOh solution**. Take 12.5 mL of your 2% EtOh solution, add 87.5 mL of saline to make a **0.25% EtOh solution**.

**Exercise 2: Cricket Cercal Ganglion Preparation**

For this exercise, you will be using measurements from the cricket cercal system to determine the effect of a drug on spiking patterns and rates.

**Note: This experiment requires crickets to be sacrificed**

1. Place your crickets in a freezer for 5 minutes. When the cricket has stopped moving and has been anaesthetized, place it on your lab bench. Take care to limit the amount of time your crickets are in the freezer, as they are more sensitive to cold than the cockroaches used previously. If 5 minutes is not enough, return the crickets back to the freezer and check on them in one-minute intervals.

2. Using your dissection scissors, decapitate the cricket at the junction between the head and the thorax. Place some petroleum jelly on the cut.

3. Place the cricket into a petri dish with a small piece of cardboard placed on the bottom. The cardboard will allow you to hold the cricket in place with the SpikerBox electrodes while applying liquids to the preparation in a clean fashion.

4. Insert your SpikerBox recording electrode through the **abdomen** of the cricket into the **cercal ganglion**. The cercal ganglion is the most posterior ganglion in the ventral nerve cord (VNC). Try to place the electrode into the center of the ganglion. Stick the ground electrode into the thorax where the head used to be. Now, using a small syringe (you can buy these at your local pharmacy), inject a few drops of **saline solution** into the abdomen of the cricket.

5. Using a toothpick or by blowing, stimulate the cerci of the cricket. This should generate a strong spiking pattern and possibly a stereotypical writhing movement of the abdomen.

6. Turn on your SpikerBox and Audacity. Connect your SpikerBox to your computer and begin recording. Plug your SpikerBox into your computer, turn it on, and start Audacity. **Adjust the Y-axis** of the recording so that the spikes observed are maximized. **Adjust the X-axis** (using the magnifying glass tool in the menu bar) so that you can differentiate bursts directly related to stimulation of the cercal ganglion.

7. If you do not observe movement or spiking in response to stimulation, be patient. It may take several minutes for the cricket’s VNC to warms to an optimal recording temperature. In this time period, activity measured from the cercal ganglion may seem quite low. If, after 5 minutes of warming, you are still unable to observe any spikes, adjust your electrodes and repeat stimulation. If you are unable to observe spikes after another few minutes of stimulation, you may need to use a new cricket. Once you have established consistent recordings, you are ready to begin observing the normal firing pattern of the cricket cercal ganglion.

**Exercise 3: Control Cercal Response**

1. You will now proceed to stimulate the cerci of the cricket by blowing from either the right or left side of the cricket. Begin recording on Audacity.

2. As one function of the cercal system is to differentiate directionality of a wind stimulus, you should be able to see differences in response to your blowing from one side versus the other. Using Table 1, record the time and duration of stimulus (blowing) and the side the stimulus was delivered (right or left). Repeat this twice for each side of the cricket.

3. Stop recording on Audacity.

4. Using the times recorded in Table 1, highlight the cercal responses to your stimulation. Amplify each of the highlighted trace using **Effects-Amplify** in the menu bar. Do not amplify the entire recording, just the cercal responses.

5. In Table 1, diagram the spiking pattern of each trace and give a brief description of any movements generated in response to your stimulation.

**Table 1. Control Cercal Responses**

|  |  |  |  |
| --- | --- | --- | --- |
| Trace | Time (Duration) | Left/Right | Pattern and Brief Description of Movement |
| 1 |  |  |  |
| 2 |  |  |  |
| 3 |  |  |  |
| 4 |  |  |  |

**Exercise 4: Testing Neuroactive Compounds**

1. Beginning with the nicotine solution, you will now test the affect of pharmacological agents on the cricket cercal response. Using a plastic pipette, remove any excess saline from the recording electrode. Begin recording with Audacity.

2. Now, using your small syringe, inject a few drops of **nicotine solution** into the abdomen of the cricket.

3. After a minute (to let your solution reach the cercal ganglion), stimulate the cerci as you did in Exercise 3. This may take several attempts. Record in Table 2 the time for two successful right and left cercal stimulations. Stop recording.

4. Using the same procedure as Exercise 3, amplify and sketch the traces of your successful stimulations. Include a brief description of any movements that appear to have been generated in response to your stimulation.

**Table 2: Effects of Nicotine on the Cricket Cercal Response.**

|  |  |  |
| --- | --- | --- |
| Trace | Time (Duration) | Pattern and Brief Description of Movement |
| Left Side 1 |  |  |
| Left Side 2 |  |  |
| Right Side 1 |  |  |
| Right Side 2 |  |  |

5. After you have finished with your nicotine solution, wash out the recording electrode with saline.

6. Repeat steps 1-5 with the Glutamate and Ethanol solutions, taking care to wash out residual solutions with saline in between trials. Record your results in Tables 3-5 below.

**Table 3: Effects of Glutamate on the Cricket Cercal Response.**

|  |  |  |
| --- | --- | --- |
| Trace | Time (Duration) | Pattern and Brief Description of Movement |
| Left Side 1 |  |  |
| Left Side 2 |  |  |
| Right Side 1 |  |  |
| Right Side 2 |  |  |

**Table 4: Effects of 0.25% Ethanol on the Cricket Cercal Response.**

|  |  |  |
| --- | --- | --- |
| Trace | Time (Duration) | Pattern and Brief Description of Movement |
| Left Side 1 |  |  |
| Left Side 2 |  |  |
| Right Side 1 |  |  |
| Right Side 2 |  |  |

**Table 5: Effects of 2% Ethanol on the Cricket Cercal Response.**

|  |  |  |
| --- | --- | --- |
| Trace | Time (Duration) | Pattern and Brief Description of Movement |
| Left Side 1 |  |  |
| Left Side 2 |  |  |
| Right Side 1 |  |  |
| Right Side 2 |  |  |

**DISCUSSION QUESTIONS**

1. Based upon your results, which pharmacological agents led to an increase in cercal activity? Was this effect consistent on both sides of the cricket? Explain.

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

2. Based upon your results, which pharmacological agents led to a decrease in cercal activity? Was this effect consistent on both sides of the cricket? Explain.

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

3. Did any pharmalogical agent affect the duration of the cercal response? If so, which? Why might this be the case?

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

4. Describe one significant difference in the spiking pattern observed between your control recordings and an experimental condition. What might these differences reflect?

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

5. Were there any differences in the movements observed between your control and experimental conditions? What might explain this?

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_